



## Article Info

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## Histopathological Effects of Aqueous *Euphorbia hirta* Extract on the Gastrointestinal Tract of Albino Rats

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*Euphorbia hirta* (Linn.), commonly known as asthma weed, is a medicinal herb widely used in traditional medicine for the management of gastrointestinal disorders, respiratory conditions, and inflammatory diseases. Despite its extensive ethnomedicinal application, there remains limited experimental evidence validating its gastrointestinal safety, particularly at the histological level. This study assessed the histopathological effects of aqueous *E. hirta* extract on the gastrointestinal tract of albino rats. Twenty adult male albino rats were randomly assigned into four groups (n = 5): Group A served as the control and received distilled water, while Groups B, C, and D were administered aqueous *E. hirta* extract orally at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight, respectively, for fourteen consecutive days. Twenty-four hours after the final administration, the animals were sacrificed, stomach and small intestine were excised, fixed, and processed histologically. A fecal occult blood test was performed to assess possible gastrointestinal bleeding, and all samples tested negative. Histology revealed normal mucosal architecture in tissue sections across all groups, except the highest dose (1000 mg/kg), where mild vascular congestion and slight epithelial folding were observed without necrosis, ulceration, or inflammatory cell infiltration. No statistically significant differences were observed when compared with the control group ( $p > 0.05$ ). These findings indicate that short-term oral administration of aqueous *E. hirta* extract does not induce significant histopathological alterations in the gastrointestinal tract of albino rats, suggesting relative gastrointestinal safety at the tested doses. Further long-term and mechanistic studies are recommended to elucidate its chronic effects.

**Keywords:** *Euphorbia hirta*; Gastrointestinal tract; Histopathology; Herbal safety.

## 1. Introduction

Natural medicine continues to play a central role in healthcare delivery, particularly in developing countries where a significant proportion of the population relies on herbal remedies for disease prevention and management (World Health Organization, 2025). Among these medicinal plants, *Euphorbia hirta* (Linn.), a member of the Euphorbiaceae family, is widely distributed across tropical and subtropical regions and is traditionally employed in the management of respiratory and gastrointestinal disorders, including diarrhea, dysentery, abdominal pain, and gastric discomfort (Chandel *et al.*, 2023; Pavithra *et al.*, 2024; Sharma *et al.*, 2025; Silalahi, 2021).

The therapeutic relevance of *E. hirta* has been linked to its diverse phytochemical composition, which includes flavonoids, tannins, phenolic compounds, alkaloids, and terpenoids (Anshu, 2024;

Sharma *et al.*, 2025; Subramani *et al.*, 2024). These bioactive constituents have been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and mucosal-protective properties. Experimental and review studies indicate that flavonoids and phenolic compounds play important roles in reducing oxidative stress, stabilizing epithelial cell membranes, enhancing mucosal defense, and supporting epithelial repair processes, mechanisms that are directly relevant to the maintenance of gastrointestinal mucosal integrity (Meda *et al.*, 2023; Sharma *et al.*, 2023).

Despite its extensive ethnomedicinal use, concerns regarding the safety profile of *E. hirta* have been reported, particularly with respect to dosage, extraction method, and duration of exposure. While several studies document beneficial gastrointestinal and antiulcer effects at moderate doses, others have

reported mild vascular or epithelial alterations at higher concentrations, indicating a dose-dependent biological response (Baba *et al.*, 2025; Senniappan *et al.*, 2024). These observations underscore the need for controlled experimental studies that directly assess tissue-level effects following oral administration.

On the other hand, the gastrointestinal tract is particularly vulnerable to xenobiotic-induced injury due to continuous exposure to ingested substances. Structural disruption of the epithelial lining, villi architecture, or mucosal vasculature may compromise digestion, absorption, and barrier function, potentially predisposing the tissue to inflammation or ulceration (Costa *et al.*, 2025; Sabucedo-Suárez *et al.*, 2025). Histological evaluation therefore remains a reliable and essential tool for assessing gastrointestinal safety following exposure to herbal preparations (Sabucedo-Suárez *et al.*, 2025).

Although pharmacological and phytochemical investigations of *E. hirta* are well documented, there remains a limited number of controlled histopathological studies that specifically evaluate the gastrointestinal safety of aqueous *E. hirta* extracts, which represent the most common form of traditional preparation. Most recent studies emphasize phytochemical profiling, antimicrobial activity, or systemic toxicity, with fewer investigations focusing on direct microscopic assessment of gastrointestinal tissues (Costa *et al.*, 2025; Elisha *et al.*, 2023; Mwalongo *et al.*, 2025).

Therefore, this study was designed to evaluate the histopathological effects of aqueous *E. hirta* extract on the gastrointestinal tract of albino rats under controlled experimental conditions. It was hypothesized that short-term oral administration of the extract at graded doses would not induce significant histological alterations in gastric or intestinal tissues, thereby providing experimental evidence to support its traditional gastrointestinal use while clarifying its short-term safety profile.

## 2. Materials and Methods

### 2.1 Experimental Animals

The study utilized twenty healthy adult male albino rats (*Rattus norvegicus*), weighing between 100 and 150 g. The animals were obtained from the Animal House of the institution and housed in clean, well-ventilated cages under standard laboratory conditions of  $25 \pm 2$  °C, relative humidity of  $55 \pm 5$  percent, and a 12-hour light and dark cycle. The rats were fed standard growers feed (Bendel Feeds Ltd., Benin City, Nigeria) and had free access to water. Animals were allowed to acclimatize for two weeks before the commencement of the experiment. Ethical approval for the study was obtained from the Institutional Animal Ethics Committee of the institution, and all procedures were conducted in

accordance with internationally accepted guidelines for the care and use of laboratory animals and the ARRIVE guidelines for animal research.

### 2.2 Collection and Identification of Plant Material

Fresh leaves of *E. hirta* were collected from the institutional parks and gardens in Edo State, Nigeria. The plant was authenticated by a qualified taxonomist, and a voucher specimen with identification number UBH-EH2025 was deposited in the institutional herbarium. The leaves were washed, shade dried for ten days and pulverized into a fine powder using a mechanical blender (Binatone BLG-450).

### 2.3 Preparation of Plant Extract and Yield Determination

Five hundred grams of the powdered plant material were soaked in one liter of distilled water for forty-eight hours with intermittent stirring. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated at 40 °C using a rotary evaporator (Buchi R-210, Switzerland). The extract was stored in a sterile airtight container at 4 °C until use. The percentage extraction yield was calculated as the ratio of the weight of the dried extract to the initial plant powder weight and expressed as a percentage, following standard procedures previously described for *E. hirta* extracts (Subramani *et al.*, 2024).

### 2.4 Experimental Design and Treatment

The animals were randomly assigned into four groups of five rats each. Group A served as the control and received distilled water. Groups B, C, and D received aqueous *E. hirta* extract orally at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight, respectively, once daily for fourteen consecutive days. The selected dose range was based on previously reported acute and sub-acute toxicity studies of *E. hirta* extracts, which demonstrated no mortality at doses up to 2000 mg/kg body weight, thereby supporting the use of graded sub-toxic doses for repeated oral administration (Baba *et al.*, 2025). The doses of used in this study therefore represent graded sub-toxic levels suitable for repeated oral administration.

### 2.5 Sample Collection and Histological Processing

Twenty-four hours after the final administration, animals were fasted overnight and anesthetized using chloroform vapor. The abdominal cavity was opened, and the gastrointestinal tract, comprising the stomach and small intestine, was excised. Tissue samples were rinsed with normal saline to remove debris and immediately fixed in 10 percent buffered formalin. Tissue processing, paraffin embedding, sectioning, and hematoxylin and eosin

staining were performed using standard histological techniques as described in recent methodological literature (Sabucedo-Suárez *et al.*, 2025). Histological evaluation was carried out by an experienced observer who was blinded to the treatment groups.

## 2.6 Fecal Occult Blood Test

Fresh fecal samples were collected from each animal prior to sacrifice. The samples were tested for the presence of occult blood using a standard fecal occult blood test kit (ACON Laboratories Inc., San Diego, CA, USA) according to the manufacturer's instructions. Briefly, a small portion of fecal material was applied to the test card, followed by the addition of the developer solution. The appearance of a blue coloration within the specified time was considered a positive result, while the absence of color change was recorded as negative.

## 2.7 Statistical Analysis

Data were expressed as mean  $\pm$  standard error of the mean. Statistical analysis was performed using one way analysis of variance followed by Tukey post hoc test. Differences were considered statistically significant at  $p < 0.05$ . Statistical analyses were conducted using IBM SPSS version 25.0.

## 3. Results and Discussion

### 3.1 Results

#### 3.1.1 Histopathological Findings

Histological examination of the stomach and small intestine was conducted following fourteen days of oral administration of aqueous *E. hirta* extract. Observations were qualitative and based on general tissue architecture, epithelial integrity, and vascular appearance.

In the control group, gastric sections showed normal histological features, including intact mucosal epithelium, well-defined gastric pits, and preserved glandular structures. Intestinal sections displayed normal villi morphology with intact epithelial lining and clearly defined lamina propria.

Rats treated with 200 mg/kg and 600 mg/kg of the extract showed gastric and intestinal histology comparable to the control group. No evidence of epithelial erosion, necrosis, ulceration, or inflammatory cell infiltration was observed. The muscularis layers remained intact, and intestinal villi appeared normal.

In the 1000 mg/kg treatment group, gastric sections showed mild vascular congestion and slight epithelial folding in some sections. However, the overall mucosal architecture remained preserved. Intestinal sections from this group also showed

normal villi structure with mild congestion and no evidence of necrosis or inflammatory infiltration.

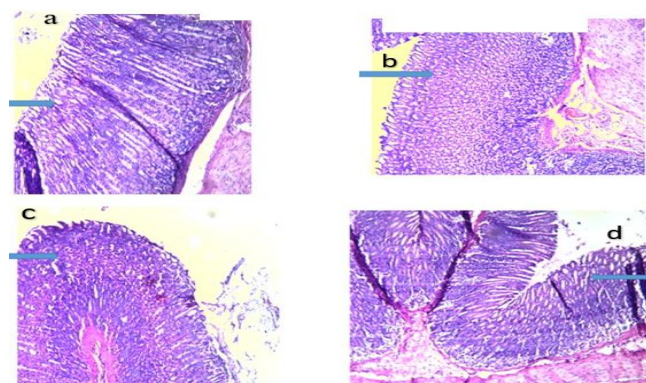
#### 3.1.2 Fecal Occult Blood Test

Fecal occult blood testing was performed to assess possible gastrointestinal bleeding. All fecal samples from control and treated animals tested negative for occult blood.

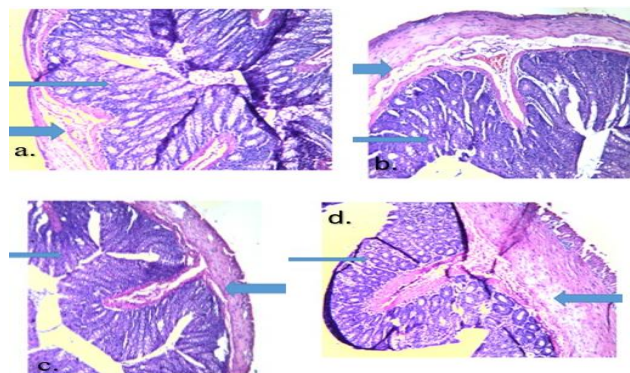
**Table 1: Summary of Histopathological Observations**

Group	Dose (mg/kg)	Gastric mucosa	Intestinal mucosa
Group A	0	Normal architecture	Normal villi
Group B	200	Normal	Normal
Group C	600	Normal	Normal
Group D	1000	Mild vascular congestion, slight epithelial folding	Mild congestion

Note: Histological assessment was qualitative and based on general tissue architecture.



**Plate 1:** Representative photomicrographs of gastric tissue from control and treated rats stained with hematoxylin and eosin (H&E) and viewed with  $\times 400$  magnification.



**Plate 2:** Representative photomicrographs of intestinal tissue from control and treated rats stained with hematoxylin and eosin (H&E) and viewed with  $\times 400$  magnification.

hematoxylin and eosin (H&E) and viewed at ×400 magnification.

### 3.2 Discussion

This study evaluated the histopathological effects of short-term oral administration of aqueous *Euphorbia hirta* extract on the gastrointestinal tract of albino rats. The findings demonstrated that administration of the extract at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight for fourteen days did not produce marked structural damage to gastric or intestinal tissues. Preservation of mucosal epithelium, glandular organization, and intestinal villi was observed across all treatment groups, with only mild vascular congestion and slight epithelial folding noted at the highest dose.

The absence of mucosal erosion, necrosis, or inflammatory cell infiltration in treated animals suggests that aqueous *E. hirta* extract does not compromise gastrointestinal histological integrity following short-term exposure. This observation aligns with recent experimental toxicity reports indicating that *E. hirta* extracts possess a relatively wide safety margin when administered at graded sub-toxic doses, with minimal tissue alterations observed at higher concentrations (Baba *et al.*, 2025). Similar mild vascular or epithelial responses at elevated doses have been documented in experimental studies and are generally interpreted as adaptive or transient physiological responses rather than overt toxicity (Pavithra *et al.*, 2024; Sakharkar *et al.*, 2024).

The maintenance of gastrointestinal architecture observed in this study may be linked to the phytochemical composition of *E. hirta*. The plant is known to contain flavonoids, phenolic compounds, tannins, alkaloids, and terpenoids, which collectively contribute to antioxidant and cytoprotective activities (Meda *et al.*, 2023; Sharma *et al.*, 2025; Subramani *et al.*, 2024). These compounds have been shown to reduce oxidative stress, stabilize epithelial cell membranes, improve mucosal microcirculation, and support epithelial repair processes, mechanisms that are directly relevant to gastrointestinal mucosal integrity (Anshu, 2024; Sharma *et al.*, 2023).

In addition to antioxidant effects, anti-inflammatory activity may also contribute to the preservation of gastrointestinal tissues observed in this study. Suppression of pro-inflammatory mediators and modulation of inflammatory signaling pathways by *E. hirta* extracts have been reported in recent investigations, which may limit tissue irritation and vascular damage following oral exposure (Amoke *et al.*, 2025; Anshu, 2024; Silalahi, 2021). These combined antioxidant and anti-inflammatory actions provide a plausible mechanistic basis for the largely preserved gastric and intestinal histology observed in the present work (Meda *et al.*, 2023).

Previous experimental studies have further demonstrated gastroprotective and antiulcer properties of *E. hirta* extracts in chemically induced injury models. Senniappan *et al.* (2024) reported preservation of gastric mucosal integrity and reduced ulcer severity following treatment with *E. hirta* extracts, while Thomas *et al.* (2025) demonstrated significant antiulcer activity of related *Euphorbia* species in experimental models. Although biochemical markers of oxidative stress or inflammation were not assessed in the present study, the histological findings are consistent with these reported protective effects.

It is important to emphasize that the present findings relate strictly to short-term exposure. While no significant histopathological alterations were observed within the fourteen-day period, prolonged exposure to high doses of plant extracts has been associated with cumulative structural or biochemical changes in other investigations (Costa *et al.*, 2025; Kgosiemang *et al.*, 2025). Therefore, the absence of overt tissue damage in this study should not be extrapolated to long-term or chronic use without further experimental validation.

#### 3.2.1 Study Limitations

This study has certain limitations that should be considered when interpreting the findings. The duration of exposure was limited to fourteen days, and the sample size was relatively small. Histopathological assessment was qualitative, and no biochemical or oxidative stress markers were measured to support mechanistic interpretation. In addition, only gastrointestinal tissues were examined, and potential systemic or reproductive effects were not assessed.

#### 3.2.2 Future Directions

Further studies are recommended to evaluate the effects of chronic and sub-chronic administration of *E. hirta* extracts, including reproductive and systemic toxicity assessments. Incorporation of biochemical assays, oxidative stress markers, and inflammatory mediators would provide deeper mechanistic insight into the observed histological findings. Such investigations would strengthen the evidence base for the safe therapeutic use of *E. hirta*.

## 4. Conclusion

This study demonstrated that short-term oral administration of aqueous *E. hirta* extract at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight for fourteen days did not result in significant histopathological alterations in the gastric or intestinal tissues of albino rats. Although mild vascular congestion and slight epithelial folding were observed at the highest dose, these changes were not associated with necrosis, ulceration, or inflammatory infiltration. The findings therefore

suggest that aqueous *E. hirta* extract does not adversely affect gastrointestinal histological integrity following short-term exposure. However, extended-duration studies, dose-escalation experiments, and investigations incorporating biochemical and mechanistic endpoints are required to establish its long-term safety profile.

### Authors' Contributions

The authors contributed collectively to the conception and design of the study. Data collection, laboratory analysis, and histological evaluation were performed by the authors. Data analysis and interpretation were carried out jointly. All authors participated in drafting and revising the manuscript and approved the final version for submission.

### Conflict of Interest

The authors declare no conflict of interest.

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